

## Cellular and Molecular Mechanisms of Learning and Memory

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The nature of the cellular basis of learning and memory remains an often-discussed, but elusive problem in neurobiology. A popular model for the physiological mechanisms underlying learning and memory postulates that memories are stored by alterations in the strength of neuronal connections within the appropriate neural circuitry. Thus, an understanding of the cellular and molecular basis of synaptic plasticity will expand our knowledge of the molecular basis of learning and memory.

The view that learning was the result of altered synaptic weights was first proposed by Ramon y Cajal in 1911 and formalized by Donald O. Hebb. In 1949, Hebb proposed his “learning rule,” which suggested that alterations in the strength of synapses would occur between two neurons when those neurons were active simultaneously (1). Hebb’s original postulate focused on the need for synaptic activity to lead to the generation of action potentials in the postsynaptic neuron, although more recent work has extended this to include local depolarization at the synapse.

One problem with testing this hypothesis is that it has been difficult to record directly the activity of single synapses in a behaving animal. Thus, the challenge in the field has been to relate changes in synaptic efficacy to specific behavioral instances of associative learning. In this chapter, we will review the relationship among synaptic plasticity, learning, and memory. We will examine the extent to which various current models of neuronal plasticity provide potential bases for memory storage and we will explore some of the signal transduction pathways that are critically important for long-term memory storage. We will focus on two systems—the gill and siphon withdrawal reflex of the invertebrate *Aplysia californica* and the mammalian hippocampus—and discuss the abilities of models of synaptic plasticity and learning to account for a range of genetic, pharmacological, and behavioral data.

The simpler model system provided by *Aplysia* has made possible the development of a cellular analog of Pavlovian conditioning, a form of associative learning, as well as habituation and sensitization, nonassociative forms of learning. In particular, studies in *Aplysia* have revealed some of the important cellular and molecular mechanisms underlying synaptic plasticity. As we will see, there have been significant advances, but it has remained difficult to relate these changes in synaptic strength to the behavior of the intact animal.

Studies of the mammalian hippocampus and its role in declarative memory have been undertaken at a variety of levels ranging from electrophysiological to pharmacological to genetic. Two major characteristics of the hippocampus—the ability of hippocampal neurons to undergo long-term potentiation (LTP), a persistent increase in synaptic strength resulting from repetitive electrical stimulation, and the existence of place cells, neurons that are active when an animal is located in a particular position in space—have been critically important in developing ideas about how the mammalian hippocampus functions in the acquisition and consolidation of spatial memories. To understand the role of synaptic plasticity in behavior, researchers have turned to genetically modified mice in an attempt to integrate information gained at the molecular and cellular levels with physiological and behavioral studies. The analysis of these mice allows researchers to test whether a particular gene product is important for LTP and provides a useful bridge between molecules and synaptic plasticity on the one hand and systems of neurons and behavior on the other. In this way, understanding the signal transduction mechanisms that underlie synaptic plasticity, combined with the use of powerful genetic technologies, has provided us with insights into the molecular basis of learning and memory.

## SYNAPTIC PLASTICITY IN *APLYSIA*

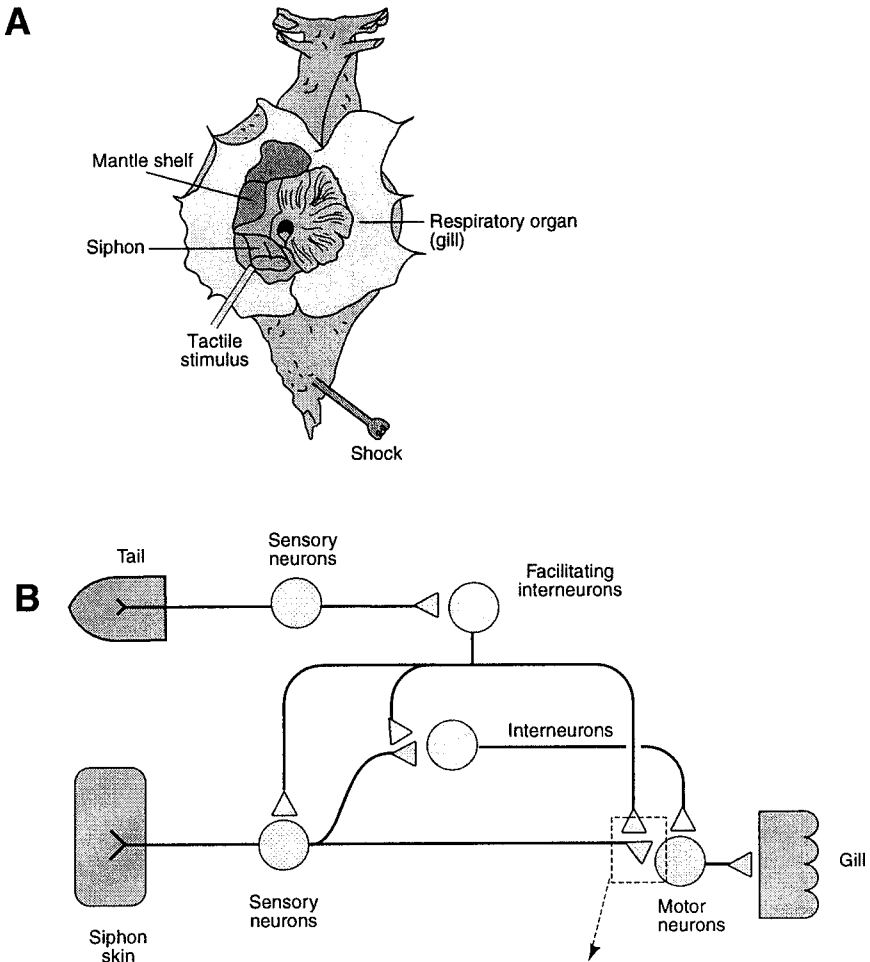
Although much modern research focuses on synaptic plasticity in mice, a large portion of our current knowledge of signal transduction pathways involved in the learning process comes from the study of the opisthobranch *Aplysia*. This organism has been an attractive model for the cellular analysis of learning for several reasons. One is that the stimulus inputs and behavioral outputs are relatively simple, yet complex enough for the systematic investigation of the mechanisms of conditioning. A second is that the neurons in *Aplysia* are large and easily identifiable from organism to organism. This large size allows recording to occur from individual neurons, and the consistency across organisms allows the neuronal circuitry to be traced more effectively than in mammalian systems. Although learning systems in *Aplysia* often are simplified to show one or two sensory neurons and a motor neu-

ron, in actuality there may be hundreds of neurons involved in the learning process (2). Even so, the ability to isolate individual neurons and synapses in culture and in the organism itself has made the study of *Aplysia* particularly fruitful for understanding the cellular mechanisms of learning. A third reason for the study of *Aplysia* is that behavioral conditioning experiments have demonstrated important similarities between conditioning in *Aplysia* and conditioning in vertebrates (3–5), although many questions remain about the extent of this generality. Because certain behavioral phenomena are common to both *Aplysia* and higher organisms, the study of *Aplysia* is predicated on the assumption that the cellular bases of these learning mechanisms can be extrapolated to synaptic plasticity in organisms with more complex nervous systems. Just as the use of model systems has revolutionized our understanding of the molecular processes underlying development, this reductionist approach to learning, pioneered by Eric Kandel and his colleagues, has been particularly fruitful in identifying molecular mechanisms of synaptic plasticity. Many discussions of *Aplysia* focus on the cellular and molecular mechanisms that form the basis of the effects of serotonin on the sensory–motor neuron coculture system. We will take a broader perspective, examining the possible roles that these mechanisms may play in behavioral processes.

### ***Nonassociative Learning in Aplysia***

Two behavioral processes that have been studied in great detail in *Aplysia* are habituation and sensitization. Research on habituation and sensitization in *Aplysia* has exploited the defensive response that occurs when an *Aplysia* is stimulated in certain ways. The components of this response are shown in Fig. 1. Shock delivered to the tail causes the tail to withdraw into the organism; shock delivered to the siphon causes the siphon and gill to withdraw. After repeated mild stimulation of either the tail or the siphon, habituation occurs, causing the withdraw response to attenuate (7). A different form of learning, sensitization, occurs when, after a single shock to the tail, the gill withdrawal response produced by subsequent mild siphon stimulation is greater than if the animal did not receive the shock (8). Both habituation and sensitization have received much attention at the cellular level because they are paralleled at the cellular level by specific changes in synaptic strength. Habituation is paralleled at the synaptic level by synaptic depression—a decrease in the amplitude of excitatory postsynaptic potentials (EPSPs; 9)—and sensitization is paralleled by facilitation—an increase in the amplitude of EPSPs (10).

In sensitization, shock to the tail increases the likelihood that a subsequent stimulation of another area, such as the siphon, will result in an increased

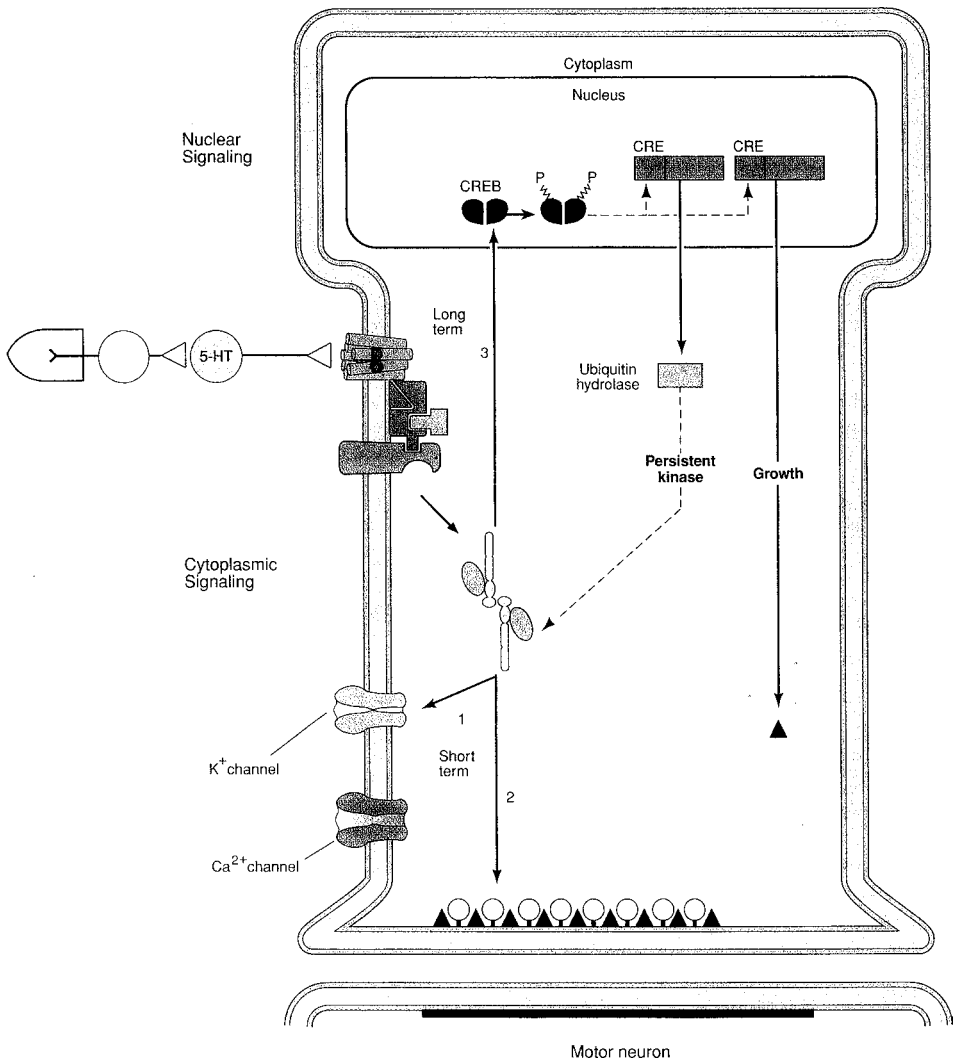


**Fig. 1.** (A) A dorsal view of *Aplysia*, showing the parts of the body stimulated in studies of habituation, sensitization, and Pavlovian conditioning. An electric shock to the tail causes the siphon to retract and the gill to withdraw. Stimulation of the siphon with a tactile stimulus causes very little response, but after this stimulation has been paired with tail shock, subsequent siphon stimulation causes a conditioned gill withdrawal response through Pavlovian conditioning. (B) Pathways involved in sensitization, habituation, and Pavlovian conditioning in the reduced *Aplysia* preparation. Stimulation of the tail by electric shock causes tail sensory neurons to excite facilitating interneurons, some of which are serotonergic. These interneurons synapse on sensory neurons from the siphon. Additional interneurons synapse on the motor neurons that control the gill withdrawal response. (Adapted from ref. 6.)

gill withdrawal response. Thus, the stimulation of the tail lowers the threshold necessary for a stimulus to elicit a response. At a cellular level, sensitization is thought to involve several steps which are diagrammed in Fig. 2 (11). Stimulation of the tail causes sensory neurons to excite interneurons, which, in turn, facilitate the release of serotonin from sensory neurons on which these interneurons synapse. Serotonin released by some of these facilitatory interneurons causes an increase in the level of cAMP in the sensory neurons, which activates cAMP-dependent protein kinase A (PKA), which phosphorylates a variety of targets, including  $K^+$  channels or proteins closely associated with them. Protein kinase C (PKC) also is activated in response to serotonin, and it may play a more important role with prolonged exposure to serotonin (11). The closure of  $K^+$  channels by PKA or PKC prevents  $K^+$  ions from escaping the cell to repolarize the cell membrane, meaning that the action potential produced by the depolarization of the neuron is broadened. As a result of broadened action potentials, more  $Ca^{2+}$  enters the presynaptic neuron, thus increasing the amount of neurotransmitter released. Increased transmitter release from the presynaptic neuron results in an increase in the amplitude of the EPSP in the postsynaptic neuron. This short-term facilitation is transient, lasting just minutes.

A longer-lasting form of sensitization occurs when stronger stimuli are used, or when weaker stimuli are applied repeatedly. This long-term sensitization results in long-term facilitation of synapses between sensory and motor neurons. Long-term facilitation differs from short-term facilitation in several key ways. First, long-term facilitation requires protein synthesis in the presynaptic neuron, whereas short-term facilitation does not (12). Second, PKA, although transiently active in short-term facilitation, is persistently active and translocates to the cell nucleus of the presynaptic neuron during long-term facilitation (13). Third, the cyclic AMP response element binding protein 1 (CREB1) is then activated in the cell nucleus, resulting in the gene transcription necessary for long-term facilitation. Recent findings suggest that another form of CREB, CREB2, may repress activation of CREB1 so that long-term facilitation does not occur as a result of mild serotonin stimulation. This repression may be released only in the presence of sufficient second-messenger activity needed for long-term facilitation to occur (14).

These cellular parallels of habituation and sensitization were of tremendous historical importance because they mapped, for the first time, behavioral learning phenomena onto a cellular process. Other forms of learning, such as Pavlovian and operant conditioning, have been more difficult to model on a cellular level because of the more complex nature of those learning processes. Because the experimenter has more control over the subject's learn-



**Fig. 2.** Molecular mechanisms that underlie sensitization in *Aplysia*. Serotonin (5-HT) binds to G protein-coupled receptors, initiating a cascade of intracellular events. G-protein-coupled receptors stimulate adenylyl cyclase to synthesize cAMP, which activates PKA. During short-term facilitation, the effects of PKA include closing  $K^+$  channels (pathway 1) and increasing transmitter release (pathway 2). Long-term effects result when PKA translocates into the nucleus and activates the transcriptional factor CREB. CREB induces the expression of effector genes which encode a variety of proteins. One class, ubiquitin hydrolases, leads to downregulation of the regulatory subunit of PKA, resulting in persistent PKA activation. The synthesis of another set of proteins ( $\blacktriangle$ ) results in growth of new synaptic connections. (Adapted from ref. 6.)

ing experience in Pavlovian paradigms compared to operant paradigms, in which a reinforcer is contingent upon an animal's response, Pavlovian paradigms have been more useful for modeling associative learning on a cellular level, although some attempts are being made at determining the pathways involved in operant conditioning (15).

### ***Associative Learning in Aplysia***

Pavlovian conditioning involves the learning of a relation between a neutral stimulus, the conditioned stimulus (CS), which on its own does not elicit a response, and an unconditioned stimulus (US), which on its own elicits an unconditioned response (UR). After CS–US pairings, the CS comes to elicit a response on its own, the conditioned response (CR). The associative properties of Pavlovian conditioning—that a previously neutral stimulus can elicit a response as a result of that stimulus being paired with a biologically relevant stimulus—make it a particularly attractive behavioral model for the study of synaptic plasticity, which involves the strengthening of synapses after a learning experience. Because of the obvious parallels between the effects of Pavlovian conditioning on behavior and of coincident synaptic activity on synaptic plasticity, the characterization of Pavlovian conditioning on the cellular level has been a primary focus of research with *Aplysia* (16,17).

Initial demonstrations of conditioning in *Aplysia* were performed in the intact animal (18,19). These experiments demonstrated Pavlovian conditioning at the behavioral level by pairing weak tactile stimulation of the siphon or mantle (the CS) with a strong electric shock to the tail (the US), which elicited a UR in the form of gill withdrawal. After repeated CS–US pairings (i.e., stimulation of the siphon or mantle followed repeatedly by the presentation of tail shock), siphon stimulation on its own elicited the conditioned gill withdrawal response. Conditioning, like sensitization and habituation, is paralleled on the cellular level by an enhancement of EPSPs in motor neurons. After conditioning, a weak stimulus that did not elicit EPSPs on its own comes to elicit EPSPs as a result of its being paired with an EPSP-evoking US.

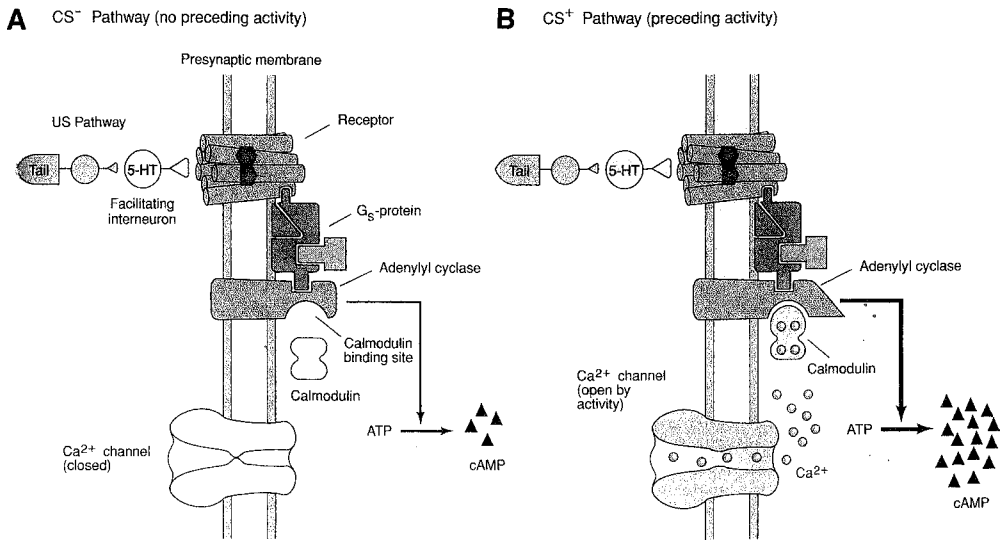
Analogues of conditioning at a cellular level have relied on the reduced preparation in which the central nervous system of the *Aplysia* is removed from the body (20). The tail remains connected to the central nervous system so that it can be stimulated with electric shock. This reduced preparation circuit is diagrammed in Fig. 1B. In this preparation, the same stimulus inputs that occur in the intact animal can be delivered and intracellular recordings can be made from various sensory and motor neurons to determine the neuronal organization of the various pathways involved in conditioning. Specifically, tail shock can be paired with stimulation of the siphon sensory neurons and

recordings can be made from synapses between sensory interneurons and motor neurons. In an even more reduced preparation, analogs of conditioning have been studied in cell culture, where treatment with serotonin substitutes for the tail shock US and spike activity in sensory neurons substitutes for the siphon stimulation CS (21). Both the reduced preparation and cell-culture studies have been used in concert with behavioral studies to develop cellular models of Pavlovian conditioning in *Aplysia*.

Cellular models of Pavlovian conditioning incorporate some of the same pathways utilized in cellular models of sensitization. The US tail shock sensorimotor pathway has been defined quite precisely in the reduced *Aplysia* preparation through some of the aforementioned studies of sensitization to tail shock. In Pavlovian conditioning, however, repeated stimulation of another sensory neuron (the CS; e.g., the siphon sensory neuron) coincident with tail shock leads to increased excitatory postsynaptic potentials in the CS–motor neuron synapse. One proposed mode of action is diagrammed in Fig. 3. The exact molecular mechanism underlying this result is not yet clear, although it is thought that the activation of the CS pathway results in  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels, which enhances transmitter release through the activation of adenylyl cyclase. The activation of the US pathway triggers interneurons to release serotonin, which binds to G-protein-coupled receptors in the CS interneurons, resulting in a potentiation of adenylyl cyclase. This coincident activation of adenylyl cyclase by G-protein-coupled receptors and  $\text{Ca}^{2+}$  leads to both short- and long-term effects. In the short term, it results in an even greater enhancement of transmitter release from the CS interneuron and increases the activation of cAMP and PKA in the presynaptic neuron. The increase in PKA activity leads to an increase in gene transcription in the presynaptic neuron, which appears to be necessary for long-term memory storage (11). Recent studies have outlined some additional mechanisms in the postsynaptic neuron that may also contribute to the learning underlying Pavlovian conditioning (17,22). The evidence for pre- and post-synaptic mechanisms will be reviewed in later sections.

If coincident activation of adenylyl cyclase in the presynaptic neuron is important, then, as in behavioral studies with vertebrates, the precise temporal relation between the CS and the US should determine the extent of conditioning. One of the cornerstones of behavioral research on Pavlovian conditioning is that the CS must precede the US for learning to occur, although the optimal delay between the CS and US varies with different preparations (23). Clark et al. (24) demonstrated that forward pairing was critical for EPSP enhancement after conditioning in the reduced *Aplysia* preparation.





**Fig. 3.** Molecular mechanisms involved in activity-dependent presynaptic facilitation. US alone trials are shown in **A**. In these trials, as in sensitization, serotonin binds to G-protein-coupled receptors, which act on adenylyl cyclase. This results in a mild increase in cAMP levels. (**B**) shows results from trials in which CS sensory neuron stimulation precedes US stimulation. CS stimulation causes an influx of  $\text{Ca}^{2+}$ , which binds to calmodulin, which in turn activates adenylyl cyclase. The coincident activation of adenylyl cyclase, caused by  $\text{Ca}^{2+}$ /calmodulin as a result of the CS and G-protein-coupled receptor binding caused by the US, results in an increased level of cAMP. (Adapted from ref. 6.)

Additionally, they demonstrated that enhancement was reduced with long CS–US intervals. It is thought that CS–US interval sensitivity occurs because the optimal window for facilitation of adenylyl cyclase in the CS sensory neurons is quite small and depends on simultaneous activation from the US pathway. For example, when the CS precedes the US by more than the optimal interval, the  $\text{Ca}^{2+}$  that enters the presynaptic neuron may have dissipated by the time the serotonin from the US pathway initiates G-protein-coupled receptor potentiation of adenylyl cyclase. Thus, with long CS–US intervals, no conditioning will occur. Similarly, backward conditioning, in which the US precedes the CS, also fails to produce learning. This may occur because the activation of adenylyl cyclase by G-protein-coupled receptors activated by the US pathway may be less persistent than that produced by  $\text{Ca}^{2+}$ /calmodulin, causing the priming of adenylyl cyclase to dissipate by the time the  $\text{Ca}^{2+}$  signal from the CS arrives (25).

Although this analysis of temporal delay in forward CS–US pairings works quite nicely in *Aplysia*, further assumptions would need to be made to extend the analysis to other Pavlovian preparations, in which the optimal conditioning interval is much longer (e.g., flavor-aversion learning [26]; auto-shaping [27]). Another complication that cellular models should address is that *Aplysia* can form associations between a context, which is always present and thus not localizable in time, and a US (3,28). More work is needed to determine how such constantly present stimuli enter into excitatory associations with the US.

Despite a large body of research examining conditioning at the cellular level, a clear understanding of the contribution of the presynaptic and postsynaptic mechanisms that drive learning remains elusive. One view of the synaptic changes required for learning holds that serotonin released from the US interneuron acts on the CS interneuron to ultimately activate PKA and CREB, causing the gene transcription necessary for long-term memory. A different view holds that postsynaptic mechanisms similar to those found in the mammalian hippocampus also are essential for long-term memory storage. Recent evidence suggests that both presynaptic and postsynaptic mechanisms are important for associative learning (21).

### *Presynaptic Mechanisms*

Much of our current understanding of presynaptic mechanisms involved in conditioning comes not only from studies of Pavlovian conditioning but also from cellular studies that attempt to model sensitization in cell culture. Although these cellular studies allow for rigorous control over the cellular processes involved in conditioning, little is known about the relevance of such studies to the behavioral phenomena they attempt to explain. Nonetheless, studies of synaptic plasticity in culture have led to several results that may help reveal synaptic mechanisms involved in Pavlovian conditioning.

The initial cellular model for Pavlovian conditioning in *Aplysia* relied on activity-dependent presynaptic facilitation (ADPF) of the sensory neuron (20). The early evidence for ADPF came from studies in which the postsynaptic neuron was hyperpolarized before the analog of conditioning began. Hawkins and colleagues (16,20) showed that long-term facilitation occurred, even when the postsynaptic neuron was hyperpolarized and thus was ostensibly unable to fire. The presynaptic mechanism responsible appeared to be a broadened action potential that occurred following cellular CS–US pairings but that did not occur when the CS and US were explicitly unpaired. However, in a voltage-clamped cell-culture preparation, Klein (29) has shown that broadening of the presynaptic action potential may not contribute significantly to the postsynaptic response.

Recent experiments have demonstrated the involvement of CREB-mediated gene transcription and PKA activity in the presynaptic but not the postsynaptic neuron during long-term facilitation. These studies have focused on posttetanic potentiation and serotonin presentation in cultured *Aplysia* neurons. PKA inhibitors reduce pairing-specific facilitation when injected into the presynaptic neuron, but not when injected into the postsynaptic neuron, suggesting that PKA is not required in the postsynaptic neuron (21). Martin et al. (30) found that long-term facilitation is specific to single axonal branches and that this facilitation relies on CREB-mediated transcription and growth of new synaptic connections exclusively at branches treated with serotonin. Interestingly, they found that presynaptic axons that were severed from their cell bodies maintained their ability to synthesize proteins in response to serotonin, suggesting that local presynaptic mechanisms were at work. These results demonstrate the importance of local synaptic action in long-term facilitation, but one needs to be cautious in extrapolating the findings of Martin et al. (30) to Pavlovian conditioning because their experiments examined the effects of repeated presentations of serotonin in culture. Similar demonstrations of the necessity for CREB-mediated presynaptic activity have yet to be performed in Pavlovian paradigms in the reduced *Aplysia* preparation. Such experiments are important, given demonstrations that conditioning in *Aplysia* is response-specific—a CS elicits different CRs depending on the US with which it is paired. A synapse-specific cellular mechanism would allow such response specificity to develop because differential local activity at the level of the synapse would enable a single neuron to be involved in multiple conditioning processes.

### *Postsynaptic Mechanisms*

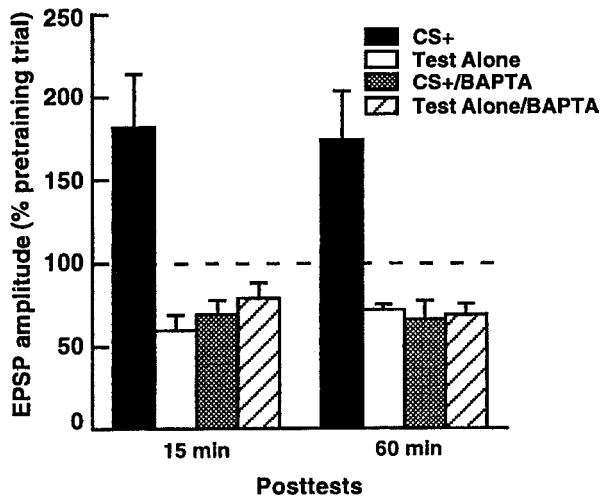
Although there is strong evidence that presynaptic mechanisms underlie learning in *Aplysia*, such evidence does not preclude postsynaptic mechanisms. The search for postsynaptic mechanisms underlying Pavlovian conditioning was motivated by Hebb's postulate and the findings that Hebbian mechanisms appear to be involved in synaptic plasticity in the mammalian brain. Such findings—including those showing that postsynaptic antagonists, such as the *N*-methyl-D-aspartate (NMDA) receptor antagonists CPP and APV, block LTP and certain forms of learning—have provided the impetus for recent research to examine the role of postsynaptic mechanisms underlying conditioning in *Aplysia*.

The initial searches for Hebbian mechanisms mediating Pavlovian conditioning in *Aplysia* failed to find evidence for postsynaptic mechanisms (16, 20). One reason for this failure might be that these experiments prevented the postsynaptic neuron from firing by hyperpolarizing it. Hyperpolarization,

while preventing the postsynaptic neuron from firing, does not necessarily prevent depolarization at the synapse, meaning that synaptic modifications may occur regardless of whether the neuron fires (2).

Recent research has attempted to gain tighter control over the postsynaptic response by blocking receptors at the synapse. Murphy and Glanzman (17) have demonstrated that the cellular analog of Pavlovian conditioning is blocked when training occurs in the presence of APV. Although they offered convincing evidence that postsynaptic mechanisms are involved in this reduced preparation analog of conditioning, APV did not completely eliminate synaptic enhancement. Additionally, the synaptic enhancement produced by unpaired CS/US presentations was similar to that produced by paired CS/US presentations when assessed 15 min following stimulation, suggesting that short-term enhancement was not dependent on the contiguous relation between the CS and US. This suggests that short-term sensitization, the increase in responding to a CS resulting simply from the presentation of a US, may not be affected by NMDA receptor antagonists. However, at 60 min, only the group that received CS-US pairings showed synaptic enhancement above basal levels. The training produced by CS-US pairings therefore led to a longer-lasting change in synaptic strength and this strength was decreased, although not eliminated, by APV, demonstrating the importance of postsynaptic NMDA receptors in conditioning.

In another test of the idea that postsynaptic mechanisms play an important role in the cellular processes mediating learning, Murphy and Glanzman (22) injected BAPTA, a  $\text{Ca}^{2+}$  chelator, into the postsynaptic motor neuron. As can be seen in Fig. 4, groups that received CS-US pairings showed an enhanced postsynaptic response at both 15 and 60 min following training. That group which received the same CS-US pairings in the presence of BAPTA showed a similar postsynaptic response to the group that received the CS stimulation only. These results again implicate NMDA receptors, because they allow  $\text{Ca}^{2+}$  into the postsynaptic neuron. One needs to be cautious, however, in attributing these results exclusively to associative mechanisms because control groups that received either random or unpaired CS-US relations were not included. Thus, it is impossible to determine the extent to which nonassociative mechanisms contributed to the results. Indeed, in their APV experiments, Murphy and Glanzman (17) showed that unpaired presentations of the CS and US enhanced EPSPs, suggesting that non-associative mechanisms may contribute to the enhancement observed with CS-US pairings. Nevertheless, these experiments by Murphy and Glanzman provide important initial evidence that postsynaptic mechanisms contribute to Pavlovian conditioning. Unfortunately, there still is no evidence that



**Fig. 4.** Results from Murphy and Glanzman (22), showing the importance of post-synaptic mechanisms in Pavlovian conditioning in the reduced *Aplysia* preparation. These data were collected at 15 and 60 min after one of four treatments. During training, the CS+/group received 12 action potentials in the sensory neuron (the CS) followed 500 ms later by the delivery of a 1-s tail nerve shock. Group CS+/BAPTA was treated identically to group CS+, except that CS-US pairings occurred in the presence of BAPTA, a  $\text{Ca}^{2+}$  chelator, in the motor neuron. Group Test Alone received only CS stimulation during training. Group Test Alone/BAPTA received CS stimulation in the presence of BAPTA in the motor neuron. (Adapted from ref. 22.)

speaks to the issue of whether these postsynaptic mechanisms are involved in conditioning at the behavioral level.

Thus, there is evidence from different experiments that both presynaptic and postsynaptic mechanisms contribute to learning. Such mechanisms have been demonstrated recently in a single set of experiments in cell coculture (21). Using serotonin stimulation as a substitute for tail shock, they found that presynaptic injection of EGTA, a  $\text{Ca}^{2+}$  chelator, or PKI6-22, a peptide inhibitor of PKA, reduced pairing-specific facilitation. Injection of PKI6-22 into the postsynaptic cell had no effect on pairing-specific facilitation, whereas postsynaptic injection of BAPTA, another  $\text{Ca}^{2+}$  chelator, reduced pairing-specific facilitation. Thus, they demonstrated that presynaptic and postsynaptic mechanisms contribute to long-term pairing-specific facilitation and that they may interact in a synergistic way, because interfering with either almost completely eliminated pairing-specific facilitation. Bao et al. (21) proposed that learning in *Aplysia* involves a hybrid mechanism consisting

of presynaptic and postsynaptic elements. One possible mechanism relies on  $\text{Ca}^{2+}$  elevation in the postsynaptic neuron, causing a retrograde messenger to be sent to the presynaptic neuron that enhances transmitter release through interaction with  $\text{Ca}^{2+}$  or cAMP.

A general finding from studies of EPSPs in cell culture and the reduced preparation is that repeated weak stimulation leads to synaptic depression—EPSPs decrease in magnitude, presumably because the postsynaptic neuron habituates to the stimulation. This suggests that in the absence of the US, or serotonin, the postsynaptic response will decrease. One thus has to be cautious in attributing increased EPSPs after CS–US pairings to an associative mechanism because dishabituation or sensitization may be contributing to the results. Indeed, Murphy and Glanzman (17) clearly showed not only that the unpaired CS/US presentations prevent the habituation obtained in CS alone procedures, but also that the unpaired presentations actually increased EPSPs above basal levels, suggesting that sensitization caused by the simple presentation of the US may contribute to the response to the CS.

Another complication that results from explicitly unpaired CS/US presentations is that the organism may learn that the CS signals the absence of the US. Behaviorally, the organism might inhibit its response in the presence of the CS as a result of these explicitly unpaired presentations because the organism learns that the CS is a signal for the absence of shock. Thus, the sensitization that has been observed when the CS and US are explicitly unpaired may be incomplete.

More experiments clearly are needed for a full understanding of the role of postsynaptic mechanisms in Pavlovian conditioning, but these recent studies make valuable progress in showing the necessity of postsynaptic mechanisms. Importantly, these findings demonstrate similarities between the synaptic mechanisms involved in conditioning in *Aplysia* and those involved in long-term potentiation in the hippocampus. Determining the ways in which presynaptic and postsynaptic neurons work synergistically to cause synaptic facilitation will be an important step in formalizing a cellular theory of learning. Additionally, more work is necessary to determine the extent to which the synaptic mechanisms and signaling molecules discovered in *Aplysia* are relevant to the behavior of the whole organism. One model system in which the relations between signal transduction and the behavior of the organism have been more firmly established is the rodent hippocampus. The ability to generate genetically modified mice has enabled researchers to make strong connections between signaling molecules, synaptic plasticity, and a range of behaviors. We now turn to the analysis of molecular and cellular mechanisms involved in hippocampus-based learning and LTP.

## SYNAPTIC PLASTICITY IN THE MAMMALIAN HIPPOCAMPUS

In humans, the medial temporal lobe system, including the hippocampal formation, is critically important for declarative memory—the conscious recollection of memories for people, places, and things (31). Beginning with studies of the patient H.M. and continuing with more recent analyses of patients with lesions restricted to the hippocampus proper, neuropsychologists have found that lesions to the hippocampus result in both anterograde and retrograde amnesia for facts and events while sparing procedural memory and motor skills.

In rodents, spatial and contextual learning are particularly well documented, and these forms of learning are sensitive to lesions of the hippocampal formation (32). Two physiological properties of the rodent hippocampus are potential cellular mechanisms underlying memory storage. First, synapses within the hippocampus undergo long-term potentiation (LTP), a form of synaptic plasticity that is thought to be involved in at least some aspects of spatial memory (33). Second, the hippocampus contains a cellular representation of space in the form of place cells that fire action potentials only when the animal is in a certain spatial location (34). Because much research has attempted to determine the relationship between LTP and learning, we will first review the evidence linking these processes and then discuss the signal transduction pathways that are important for long-lasting forms of synaptic plasticity and long-term memory storage.

Synaptic plasticity, the change in the strength of synaptic connections in the brain, is thought to underlie memory storage and the acquisition of learned behaviors. One intensely studied form of synaptic plasticity is LTP, a persistent, activity-dependent form of synaptic enhancement that can be induced by brief, high-frequency stimulation of hippocampal neurons (33). LTP, first described in detail by Bliss and Lomo in 1973, can be measured in hippocampal slices or in awake, behaving animals, where it can last for several weeks. The duration of LTP makes it an attractive model for certain types of long-term memory in the mammalian brain. In addition, LTP has other properties, including *associativity*, by which LTP induction at one synapse may be regulated by other inputs, *cooperativity*, which refers to the observation that a greater stimulus intensity will produce greater LTP, and *pathway specificity*, which refers to the observation that only synapses active at the time of LTP induction will be potentiated. These elements of LTP make it an ideal mechanism for memory storage from a computational perspective. On a molecular level, these properties derive, in large part, from the properties of a specific type of postsynaptic receptor for glutamate, the NMDA receptor, which serves as a molecular coincidence detector.

For the past 25 years, LTP has been studied as a potential cellular model of memory storage—a representative of the types of synaptic plasticity that may occur naturally during learning. There are four elements that comprise the basis of the hypothesis that spatial information is stored as activity-dependent alterations in synaptic weights in the hippocampal formation: *enhancement*, the idea that increased synaptic strength should accompany learning; *saturation*, the proposal that learning impairments should be observed after the saturation of LTP in hippocampal circuits; *blockade*, the hypothesis that spatial learning should be disrupted after the blockade of hippocampal LTP; and *erasure*, the idea that erasure of LTP should result in forgetting.

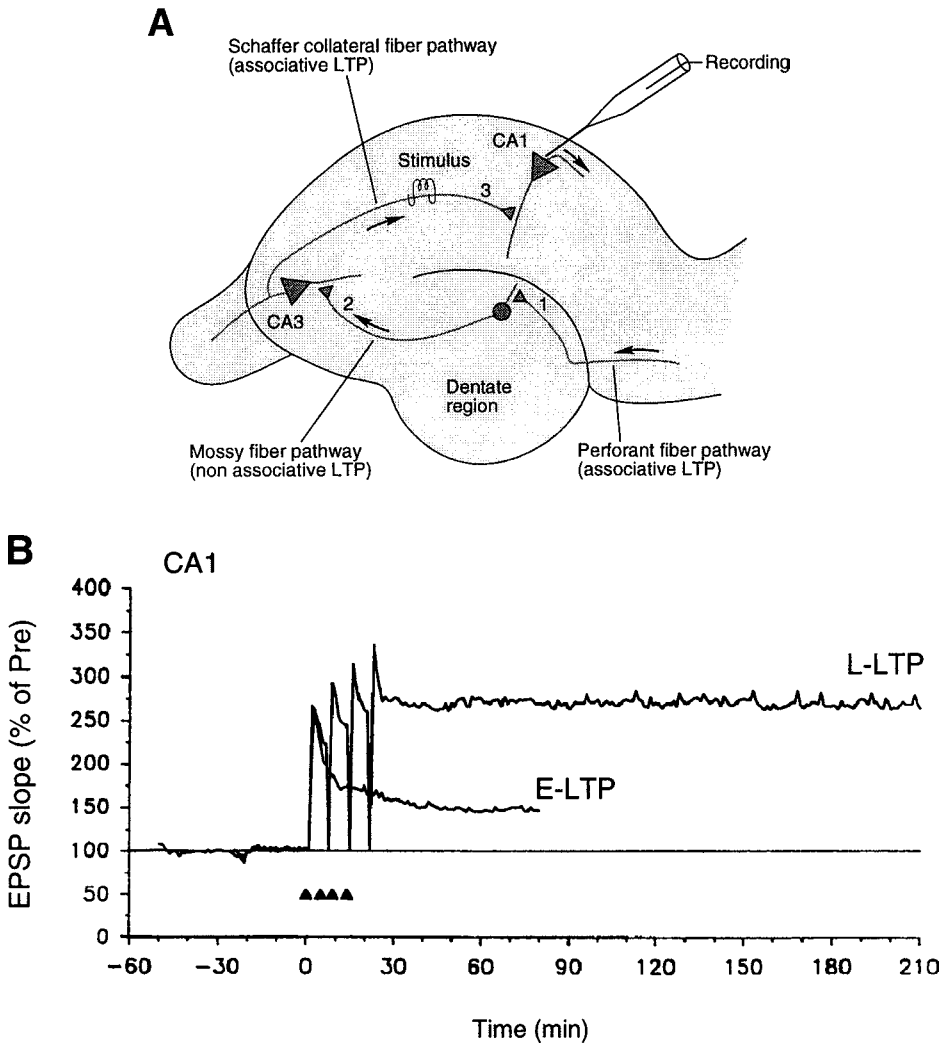
We will explore the experimental approaches that have been followed to investigate the relationship between LTP and learning. In particular, if LTP and learning recruit the same underlying physiological and cellular mechanisms, then modifying the ability of hippocampal synapses to undergo LTP should alter learning. In turn, learning should modify synaptic strength.

LTP experiments have focused particularly on the three major pathways in the hippocampal trisynaptic circuit: the perforant pathway between the entorhinal cortex and dentate gyrus granule cells, the mossy fiber pathway between dentate gyrus granule cells and CA3 pyramidal cells, and the Schaffer collateral pathway between CA3 and CA1 pyramidal cells (Fig. 5A). For many of these experiments, investigators have studied synaptic transmission in the perforant path, where recordings can be easily made in vivo in awake, behaving rodents.

### *Experience-Dependent Changes in Synaptic Strength*

If synaptic plasticity underlies learning, then an increase in synaptic strength would be expected to accompany behavioral training. Early experiments to test this idea observed an increase in the size of the population spike and fEPSP in the dentate gyrus after the exposure of rats to a spatially complex environment (35). As investigators attempted to extend this observation to hippocampus-dependent tasks such as the Morris water maze, they found, paradoxically, that spatial training in the Morris water maze resulted in a decrease in the size of the fEPSP as measured in the dentate gyrus. In exploring this observation, Moser et al. (36) found that striking changes in brain temperature occurred during different tasks. An increase in hippocampal temperature, as observed during active exploration or treadmill running, was correlated with an increase in fEPSP slope. During training in the Morris water maze, in which the water temperature is typically cooler than body temperature, the brain temperature drops and the fEPSP slope decreases.





**Fig. 5.** (A) The major areas and pathways in the hippocampus. Input from the entorhinal cortex is relayed to the dentate gyrus via the perforant pathway. The mossy fiber pathway relays the signal from the dentate gyrus to area CA3. The Schaffer collateral pathway relays the signal from area CA3 to area CA1. In the example shown, LTP is induced by the stimulating electrode in the Schaffer collateral pathway and is recorded by the recording electrode in area CA1. (B) Different phases of LTP: E-LTP occurs after one tetanus (one filled triangle); L-LTP occurs after four tetani (four filled triangles). E-LTP lasts about 1 h, but L-LTP can last for up to 8 h in hippocampal slices. In contrast to E-LTP, L-LTP requires translation, transcription, and protein synthesis. (Adapted from ref. 6.)

Further, changes in synaptic strength may also occur as a result of the stress that accompanies behavioral training (37). Thus, the differentiation of learning-related changes in synaptic strength as measured *in vivo* from the large nonspecific changes that are produced during the performance of a task has proven difficult, especially for hippocampus-dependent tasks. If only a small number of synapses increase in strength during learning, or if some synapses are potentiated while others are depressed, it may be difficult to accurately measure changes in synaptic strength that result from learning. Indeed, after the temperature component of the field potential changes that occur during training is subtracted, only a short-lasting (15–20 min) increase in the fEPSP and population spike is found to occur during learning (38). These experiments have focused on the idea that learning alters baseline-evoked synaptic transmission, but an alternative possibility, which has been the focus of only a few studies, is that learning alters synaptic plasticity, modulating the ability of synapses to undergo LTP (39).

Although the demonstration of LTP-like enhancements in synaptic strength in the hippocampus in the behaving animal during learning has been elusive, studies of cued fear conditioning, a form of Pavlovian conditioning in which an animal learns to fear a previously neutral tone as a result of its association with an aversive stimulus such as footshock, have revealed that learning is indeed associated with an increase in synaptic strength in the appropriate neural circuit (40,41). Information about the CS (tone) and the US (footshock) converge in the lateral nucleus of the amygdala. LTP, when induced by electrical stimulation in the connections between the auditory thalamus and the lateral nucleus, increases the response of neurons in the lateral nucleus to auditory stimulation. Thus, responses to natural stimuli in this system can be modulated by LTP (42).

By monitoring extracellular potentials in the lateral nucleus in response to tones, Rogan et al. (41) demonstrated that cellular responses to the CS increased during the period of paired presentation of the CS and US as the animal acquired the fear response to the tone. Importantly, extinction of the fear response produced by the nonreinforced presentation of the tone caused the auditory-evoked potential to return to baseline. It is striking that the increased synaptic response to the CS parallels learning, but it has not been demonstrated that this increase is specific to the CS, nor is it clear exactly where in the auditory processing circuitry the increase occurs. The control used in these experiments was the explicitly unpaired presentation of the tone and shock, resulting in a decreased auditory-evoked potential following training, perhaps because the animal learns that the shock is not signaled by the tone as a result of this training procedure. Thus, unpaired training is not a “neutral” control protocol and may involve some learning. A better

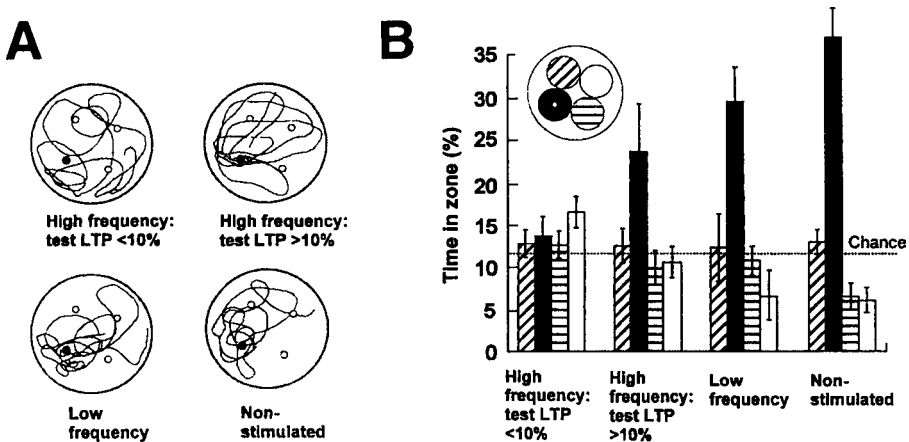
control might utilize a random pairing protocol in which the CS provides no information about the US (43). Further, it remains to be explored whether these behavioral changes in synaptic strength are, like fear conditioning, dependent on NMDA receptor activation.

### ***Behavioral Effects of LTP Saturation***

If learning is the result of changes in the same sets of synapses as those modified by LTP, then the induction of LTP by tetanization—brief trains of high-frequency stimulation in presynaptic neurons—would be predicted to alter learning. In physiological studies, the induction of LTP blocks or occludes further potentiation, suggesting that tetanization of synaptic circuits within the hippocampus would impair learning. Many computational models assume that information is stored in the pattern of synaptic weights rather than in the absolute strength of synaptic connections (44). By this view, then, the uniform saturation of synapses would block further learning by equalizing synaptic weights.

In the first saturation study published, McNaughton et al. (45) implanted electrodes bilaterally to stimulate the perforant pathway. Animals that received bilateral tetanic stimulation had deficits in the Barnes circular platform maze, a spatial task in which animals must learn the location of an escape hole on a circular maze. These observations were extended to the Morris water maze, in which tetanized rats exhibited a deficit in their ability to learn the location of a hidden platform, a task dependent on an intact hippocampus (46). These initial experiments provided powerful support for the idea that interfering with plasticity in specific hippocampal pathways could disrupt learning, but several attempts to replicate these observations were unsuccessful (47). Although these failures to replicate the saturation experiments have called into question the idea that synaptic plasticity is used during the learning of spatial tasks, several alternative explanations have been developed to explain why LTP saturation does not, in some instances, block learning. LTP induction techniques, for example, may not activate all of the fibers required for spatial learning. Indeed, tetanization at a single stimulation site fails to saturate the entire perforant path (48) and lesion experiments have revealed that learning can be supported by just a small fraction of the hippocampus (49).

A recent experiment by Moser et al. (50) has revisited this question of the impact of LTP saturation on spatial learning in an ingenious way, and their results suggest that saturation does indeed impair spatial learning in the Morris water maze. To strengthen the experimental design and to increase the percentage of the synapses that were saturated, they made three modifications. First, based on their previous observation that unilateral hippocampal lesions



**Fig. 6.** Effects of saturation of LTP on behavior. Rats received either high-frequency tetanization, low frequency tetanization, or no tetanization. Rats that received high-frequency tetanization were divided into two subgroups: those that showed less than 10% LTP (saturated) on the test and those that showed greater than 10% LTP (nonsaturated) on the test. **(A)** Sample paths from Morris water maze probe trials, in which the hidden platform was removed. **(B)** Time spent in different zones in the pool. Filled bars indicate the target zone (the zone in which the platform was located during training). Error bars indicate standard errors of the mean and the dotted line indicates chance level. (Adapted from ref. 50.)

do not impair performance in the water maze, they reduced the volume of functional hippocampal tissue by unilaterally lesioning the hippocampus. Second, to saturate a greater percentage of perforant path synapses, two bipolar stimulating electrodes were placed on each side of the angular bundle, the tract that carries the perforant path fibers into the hippocampus. Using these electrodes, multiple “cross-bundle” episodes of tetanic stimulation can be applied, thus saturating LTP in the maximal number of synapses. Finally, they monitored potentiation by implanting a third stimulating electrode.

After saturating LTP with five episodes of cross-bundle tetanization, animals were trained in the spatial version of the Morris water maze. Moser et al. (50) found a wide range of spatial learning in the rats following tetanization. Unlike previous studies, however, they determined the extent of saturation by measuring the amount of residual LTP using a “naive” test electrode and thus were able to correlate performance in the water maze with levels of residual LTP. Strikingly, they found that impairments in performance correlated with lower levels of residual LTP. Thus, animals in which LTP was saturated were poor learners, whereas animals in which LTP was unsatur-

ated were good learners, in support of the hypothesis that synaptic plasticity underlies learning (Fig. 6). This positive result showing that LTP saturation impairs spatial learning, however, might be due to nonspecific effects of tetanization on basal synaptic transmission and thus does not prove that synaptic plasticity underlies learning. Thus, it will be particularly important to determine if the group with little residual LTP regains spatial learning ability as the level of saturation decays over time and the ability to induce LTP is restored. This reversibility might help address some concerns about nonspecific side effects of high-frequency tetanization. Further, it will be interesting to determine the overall relationship between residual LTP (percent saturation) and spatial learning to see if they are correlated across a range of impairments.

### *Pharmacological and Genetic Blockage of LTP*

Some of the strongest evidence linking LTP and learning is derived from experimental approaches using pharmacological or genetic manipulations that modulate LTP. By determining the effects of these manipulations on learning and memory, investigators have been able to correlate alterations in synaptic plasticity with behavioral impairments in hippocampal function. With the development of techniques such as targeted gene ablation and transgenesis, it has become clear that mice offer a superb genetic system for determining the role of individual gene products in synaptic plasticity and memory storage. The analysis of genetically modified mice allows researchers to test whether a particular gene product is important for LTP, and the use of this genetic approach to study neuronal physiology and behavior has drawn attention to the correlation between memory storage and hippocampal LTP.

As mentioned earlier, LTP exhibits synapse specificity and associativity because it occurs only when presynaptic activity is paired with postsynaptic depolarization. On a molecular level, these characteristics can be explained by the fact that the NMDA subtype of glutamate receptor is both ligand gated and voltage sensitive. Importantly, many forms of hippocampal LTP share a dependence on NMDA receptor function with many forms of spatial memory.

To explore the role of NMDA-receptor-dependent synaptic plasticity in spatial learning, Morris and co-workers (51,52) infused APV into the cerebral ventricles of rats and examined their performance in the Morris water maze. APV treatment resulted in longer latencies to find the hidden platform during training and little spatial specificity of search in a probe trial, during which the platform was removed from the pool. Although these experiments provide strong evidence in support of the link between LTP and learning, several caveats have emerged since these studies were published.

First, several forms of synaptic plasticity—such as long-term depression (LTD), a long-lasting decrease in EPSPs—depend on NMDA receptor function (53), so it is difficult to make direct connections between any one form of synaptic plasticity and spatial learning. Second, APV may be affecting processes in brain regions other than the hippocampus when administered in this way. These processes may include modulation of sensory input, modifying anxiety, and altering motor abilities (39). Third, the relationship between NMDA receptor function and spatial learning has become complicated by the observation that spatial learning is NMDA-receptor independent if animals are first pretrained in the water maze in a different environment (54,55).

The strongest correlation between LTP and spatial memory comes from the study of mice in which the R1 subunit of the NMDA receptor was deleted in a regionally restricted fashion, only in hippocampal area CA1 (53). This study is particularly important because it underscores the power of molecular approaches to study learning and memory. Conventional knockouts of the gene encoding the R1 subunit of the NMDA receptor were lethal, so Tsien and co-workers turned to a conditional knockout approach using Cre recombinase. They used the calcium/calmodulin-dependent protein kinase II $\alpha$  (CaMKII $\alpha$ ) promoter to express Cre recombinase postnatally in neurons within the forebrain. To selectively delete the NMDA R1 gene, they inserted lox P sites, which are recognized by Cre recombinase, into the NMDA R1 locus. Using this approach, they achieved both temporal and regional restriction, knocking out NMDA receptor function only in hippocampal area CA1, a result not possible with pharmacological approaches. Thus, this genetic approach may overcome some of the difficulties encountered in the above-described pharmacological studies.

These mutant mice lacking NMDA receptor function only in hippocampal area CA1 have impaired Schaffer collateral LTP and deficits in spatial memory, providing evidence supporting a selectively important role for hippocampal area CA1, as suggested earlier by the study of the patient R.B. by Squire and colleagues (56). However, these mutant mice also have impaired LTD (53); thus, the nature of the synaptic plasticity deficit underlying their behavioral abnormality is unclear. In addition, it will be important to determine the effect of spatial and nonspatial pretraining on the impairments observed in these mutants in the spatial version of the water maze. It also will be important to explore whether these NMDA R1 transgenic mice exhibit deficits in nonspatial forms of hippocampus-dependent learning such as contextual fear conditioning (57,58) and olfactory-based tasks such as social transmission of food preferences (59,60). Deficits in these nonspatial tasks would implicate NMDA receptors in area CA1 in a variety of learning tasks.

### ***Protein Kinase A, Long-Term Memory, and the Late Phase of LTP***

Like the study of mice lacking the R1 subunit of the NMDA receptor only in hippocampal area CA1, the study of other genetically modified mice has focused on the early, transient phase of LTP (E-LTP) in area CA1 that lasts about 1 h. These studies have shown that genetic manipulation of any one of several kinases interferes with not only E-LTP but also short-term memory (61,62). The study of amnesiac patients and experimental animals has revealed, however, that the role of the hippocampus in memory storage extends from weeks to months (63,64), suggesting that longer lasting forms of hippocampal synaptic plasticity may be required.

Long-term potentiation in the CA1 region of hippocampal slices, like many other forms of synaptic plasticity and memory, has distinct temporal phases (65), as shown in Fig. 5B. In contrast to E-LTP, the late phase of LTP (L-LTP) lasts for up to 8 h in hippocampal slices (66) and for days in the intact animal (67). Long-term memory storage, in contrast to short-term memory storage, is sensitive to disruption by inhibitors of protein synthesis (68), and L-LTP in the CA1 region of hippocampal slices, unlike E-LTP, shares with long-term memory a requirement for translation and transcription (66,69–71).

Although extensive information is available about E-LTP and its relationship to behavior, less is known about the behavioral role of L-LTP. Pharmacological experiments have suggested that PKA plays a critical role in L-LTP (66,70). One of the nuclear targets of PKA is CREB (72), and CRE-mediated gene expression is induced in response to stimuli that generate L-LTP (73). Behavioral studies of mice lacking the  $\alpha$  and  $\Delta$  isoforms of CREB have suggested that this transcription factor plays a role in long-term memory storage, but the relationship between these memory deficits and L-LTP is unclear, because a deficit in LTP is observed during E-LTP following a single stimulus train (74,75). Moreover, because CREB is a multifunctional transcription factor that can be activated by second-messenger systems other than PKA, including CaM kinases and the MAP kinase pathway (76), these data on CREB knockout mice do not define a role for PKA in long-term memory.

To explore the role of PKA in long-lasting forms of synaptic plasticity and behavioral memory, Abel et al. (77) used transgenic techniques to reduce PKA activity in a specific subset of neurons within the mouse forebrain by using the CaMKII $\alpha$  promoter to drive expression of R(AB), a dominant negative form of the regulatory subunit of PKA. R(AB) carries mutations in both cAMP binding sites and acts as a dominant inhibitor of both types of PKA catalytic subunits (78–80). The transgenic approach is more spatially and temporally restricted than is the conventional gene knockout approach,

thereby allowing for a more direct correlation between a behavioral deficit and synaptic physiology in the adult brain. Transgenic techniques are particularly powerful for the study of signaling molecules, such as PKA, that are encoded by multiple genes. Appropriately designed dominant-negative mutants can inhibit multiple related gene products simultaneously, an effect that cannot be obtained through conventional single-gene knockouts (77). Further, appropriately designed constitutively active mutants can be used to activate an endogenous signaling pathway.

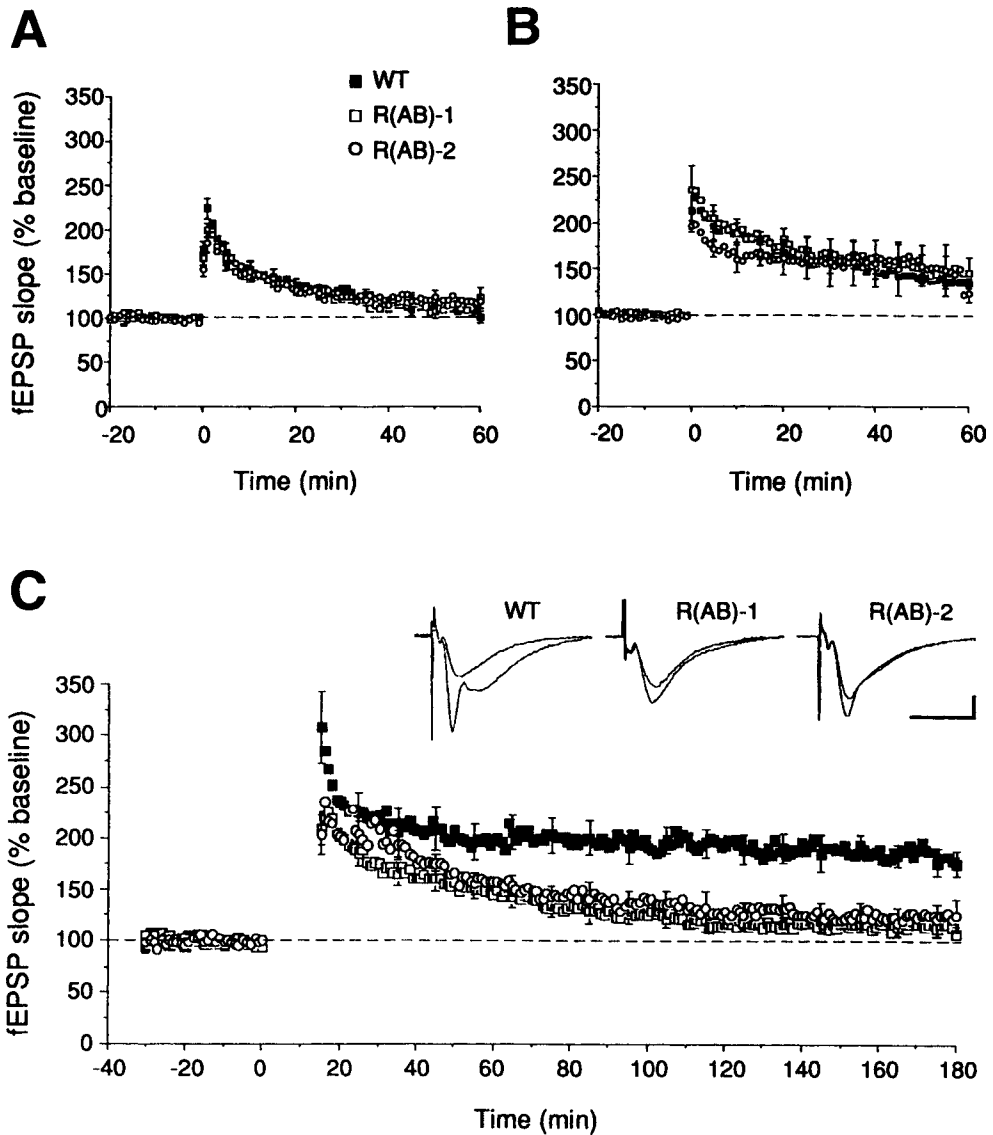
R(AB) transgenic mice have reduced hippocampal PKA activity, as well as impairments in L-LTP induced by repeated tetanization (four 100-Hz trains, 1-s duration, spaced 5 min apart) of Schaffer collateral pathway slices (77). E-LTP induced by one or two stimulus trains is unchanged in the R(AB) transgenics, suggesting that L-LTP, unlike E-LTP, requires PKA and recruits distinct signaling pathways immediately following tetanization (Fig. 7).

For the behavioral analysis of hippocampal function, R(AB) transgenic animals have been tested in the hidden platform version of the Morris water maze task (81). Transgenic animals improved during training, indicating that they learned the task, but when tested for memory in a probe trial, transgenic animals exhibited spatial memory deficits (77). The Morris maze task requires repeated training over several days and does not, therefore, provide the temporal resolution necessary to distinguish between different phases of memory storage. Because L-LTP and long-term memory share a requirement for protein synthesis, one might predict that the R(AB) transgenics, which have a L-LTP deficit, would have normal short-term but defective long-term memory.

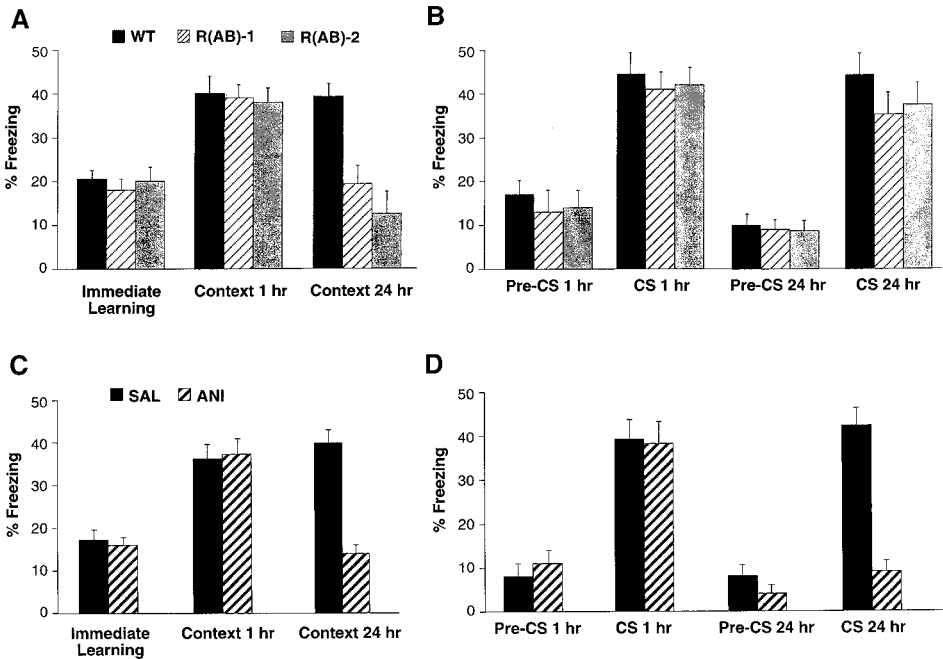
To define more precisely the time course of the memory deficit in R(AB) transgenics, contextual and cued fear-conditioning tasks, in which learning can be accomplished by a single training trial, have been used (57,58). The R(AB) transgenics exhibited normal short-term memory, consistent with normal E-LTP, but deficient long-term memory for contextual fear conditioning (77), a task that is sensitive to hippocampal lesions (57,58,82,83). The time-course of the memory deficit of R(AB) transgenics in contextual fear conditioning parallels that of wild-type animals treated with the protein synthesis inhibitor anisomycin (Fig. 8). By contrast, the long-term memory for cued conditioning, a task sensitive to amygdala lesions but insensitive to hippocampal lesions, is not disrupted in R(AB) mice (Fig. 8). Importantly, R(AB) transgenic mice also showed normal long-term memory in the conditioned taste-aversion task (77), a task which is sensitive to amygdala lesions (84).

One concern about using genetically modified mice is that any observed behavioral effects may be the result of developmental effects of the transgene rather than a direct, acute effect of the transgene on memory storage in



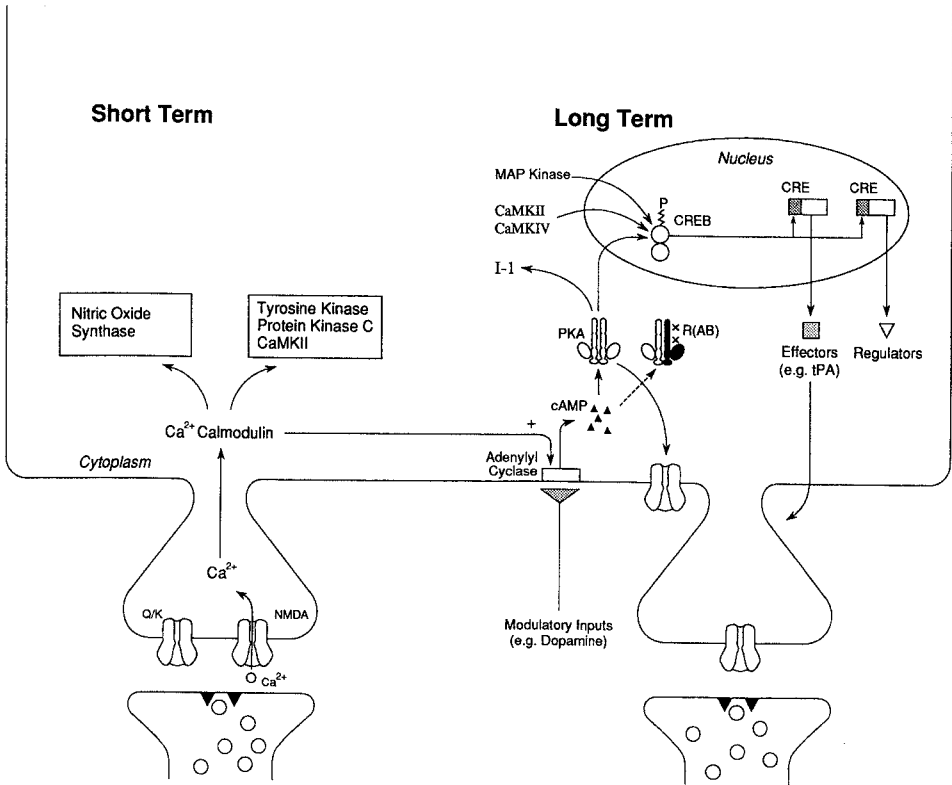


**Fig. 7.** LTP deficits in R(AB) transgenic mice. LTP was reduced in two different lines of transgenic mice (R[AB]-1 and R[AB]-2) following four 100-Hz trains (1-s d, 5 min apart) of stimulation. After about 2 h posttetanus, potentiation in the R(AB) mice returned to near-baseline levels but remained robust in wild-type mice. Sample fEPSP traces also are shown. They were recorded in area CA1 in wild-type, R(AB)-1, and R(AB)-2 slices 15 min before and 180 min after the four tetanic trains. Each superimposed pair of sweeps was measured from a single slice. Scale bars: 2 mV, 10 ms. (Adapted from ref. 77.)



**Fig. 8.** Fear conditioning in R(AB) transgenics and anisomycin-injected wild-type mice. All mice received one CS–US pairing and then were tested immediately, 1 h, and 24 h after training. (A) R(AB) mice showed a deficit in long-term but not short-term memory for contextual fear conditioning. (B) R(AB) mice showed no deficits in cued fear conditioning. (C) Anisomycin disrupted long-term but not short-term memory for contextual fear conditioning. (D) Anisomycin disrupted long-term but not short-term memory for cued fear conditioning. (Adapted from ref. 77.)

the adult. To provide a complementary way to study the role of PKA in memory storage and to address concerns about potential developmental effects of the R(AB) transgene, a pharmacological approach was taken using Rp-cAMPS, a membrane-permeant, phosphodiesterase-resistant inhibitor of PKA (85). Intraventricular injection of Rp-cAMPS selectively affects long-term memory for contextual fear conditioning with a time-course similar to that seen in R(AB) transgenic animals or in wild-type mice after the administration of anisomycin (86). The long-term memory deficits that occur in R(AB) transgenic mice and in wild-type mice after pharmacological inhibition of PKA demonstrate that the PKA pathway plays a crucial role in the hippocampus in initiating the molecular events leading to the consolidation of short-term memory into protein synthesis-dependent long-term memory in mammals. The molecular events involved in the short- and long-term synaptic plasticity that are thought to underlie memory are diagrammed in Fig. 9.



**Fig. 9.** Molecular schematic of LTP. In the absence of activity, the NMDA receptor is blocked by  $Mg^{2+}$ . This  $Mg^{2+}$  is expelled when non-NMDA (Q/K) receptors open and depolarize the membrane. Coincident binding of L-glutamate to the NMDA receptor allows  $Ca^{2+}$  influx, which activates several kinases, including tyrosine kinase, protein kinase C, and CaMKII. The activation of these kinases may be required for E-LTP, which corresponds to short-term memory. L-LTP, which corresponds to long-term memory, requires PKA activation and protein synthesis. When  $Ca^{2+}$ /calmodulin binds to adenylyl cyclase, cAMP levels rise, causing an activation of PKA, which phosphorylates ion channels, protein phosphatase inhibitor-1 (I-1), and nuclear targets such as CREB. CREB then activates effector genes encoding proteins that are necessary for alterations in synaptic strength and perhaps synaptic growth, such as tissue plasminogen activator (tPA). (Adapted from ref. 11.)

These studies underscore the crucial interplay between pharmacological and genetic studies that are greatly expanding our knowledge of the molecular basis of synaptic plasticity, learning, and memory. By determining the signal transduction pathways critically important for long-lasting forms of synaptic plasticity, the sophisticated tools of mouse genetics can then be

used to modify this signal transduction pathway *in vivo*. These functional experiments provide a rigorous test of the role of gene products, such as PKA, in learning and memory.

## ***Hippocampal Place Cells***

### *Basic Properties*

The research described in the previous sections suggests that LTP may play a critical role in the synaptic plasticity underlying memory storage. Deficits in hippocampal LTP often are accompanied by deficits in spatial learning and in learning about the identities of contexts. This implies that one function of LTP in the hippocampus may be to mediate configural representations of multiple environmental stimuli (87). In addition to undergoing LTP, another characteristic of neurons in the hippocampus is that some of them are place cells that respond selectively to particular locations in the environment (88). The discovery of these place cells was an important step in placing physiological reality on theories of cognitive mapping, which was thought to be necessary to navigate the environment (88,89). Because these cells seemed to be located almost exclusively in the hippocampus, their discovery was an important modern development in driving theories of hippocampal function. The recent emphasis on research with genetically modified mice has allowed strong links to be made among the molecular characteristics of place cells, synaptic plasticity, and behavior. The correlation between place cell function, synaptic plasticity, and spatial learning has generated specific hypotheses about the role of LTP in spatial learning, ranging from being important for the establishment of place fields to modifying synaptic strength both among place cells themselves and between place cells and other brain regions.

The majority of place cells in the brain are pyramidal cells found in areas CA1 and CA3 of the hippocampus. This does not mean that all pyramidal cells in the hippocampus are place cells; 70% to over 90% of pyramidal cells are place cells, and approximately half of them act as place cells in a given environment (90); nor does it mean that there are no place cells outside of the hippocampus proper (91). Additionally, the other major class of neurons in the hippocampus, theta cells, also code some spatial information (92). Nevertheless, most of the work on place cells has focused on the pyramidal cells in areas CA1 and CA3, not only because these cells clearly function to a large degree as place cells but also because these areas of the hippocampus have been shown to be important in learning.

The most fundamental property of place cells is their place-specificity—a given cell fires only when the organism is in a certain location in the envi-

ronment, although a small portion of place cells fire in more than one location in the same environment (93). Place-specificity is observed after a single exposure to an environment (88), and once established, place-specificity is stable for at least several months (94). That it is established during the first exposure suggests that the initial formation of place fields is an unconditioned response that occurs to the environment. These place fields are dependent on cues in the environment; when environmental cues shift, place fields shift accordingly (34). Learning may occur with prolonged or multiple exposure as the organism processes more and more about the stimulus environment. Similarly, although rewards are not necessary for the formation of place fields, an animal's ability to remember the spatial location of a reward may depend on associations between place fields and reward centers in the brain.

In contrast to simple sensory mapping, such as that which occurs in retinotopic mapping, place cell mapping does not occur topographically; adjacent cells do not represent adjacent points in the environment. Indeed, there is little correlation between the location of place fields and the location of place cells in the hippocampus; two physically adjacent place cells may fire at opposite points in the environment and two place cells located far apart within the hippocampus may fire at similar locations in the environment (34). Additionally, a given place cell may fire differently depending on the environment, resulting in the same place cells potentially being involved in distinct cognitive maps in different environments. The system properties that lead to the unique configural representations required for each environment remain unknown, although connectionist models have been developed to provide a theoretical basis for such representations (44,95).

Perhaps the most interesting and fundamental issue that has yet to be resolved about place cells is how these cells contribute to learning. Many place cell experiments have recorded from rats exploring different environments, in which there is neither a reward to be found nor an aversive situation to escape. Place fields form in these environments independent of the organism's having learned anything about the biological significance of a given environment. These experiments speak to the existence of place cells but are quiet on the issue of the involvement of place fields in tasks that require learning about spatially distributed stimuli. It is clear from maze experiments, in which animals must learn the relation between distal stimuli and a reward, that place fields are involved in locating rewards. O'Keefe and Speakman (96) recorded place fields from rats searching in a four-arm maze for a food pellet. They trained rats to locate a food reward by learning the relation between environmental cues and the reward. Some of their most interesting results occurred on trials in which the cues were removed. When

the rat made an incorrect choice in the absence of cues, the place cells fired as if the rat had made the right choice. This suggests that place fields may be used to navigate through space to find a reward and that the firing of place fields is controlled not only by environmental cues but also by the expectations of the organism. Determining the mechanisms that incorporate place cells into such goal-directed behavior will be an important step in forming an integrative theory of place cell function.

### *Hippocampal Place Cells and LTP:*

#### *Pharmacological and Genetic Approaches*

The pharmacological and genetic approaches aimed at studying the relation of LTP to spatial memory, which have been outlined in previous sections, also have been applied to study the relation between place cells, synaptic plasticity, and memory storage. Recent studies have demonstrated correlations among place cell function, LTP, and spatial learning. These experiments have shown a direct correlation between synaptic functioning and spatial learning—LTP deficits often are accompanied by spatial learning deficits in the Morris water maze and alterations in place cell properties (53,90,97–99). These correlations suggest that LTP likely is involved in place cell function somewhere along the path leading to spatial memory. The precise role of LTP in this process remains unknown, but there are many possibilities. For example, LTP may be involved in the formation, maintenance, stability, or spatial selectivity of place cells. Additionally, LTP may play a role either in establishing connections between place cells themselves or in establishing connections between place cells and other brain regions, such as those involved in learning behavioral responses or those involved in processing rewards.

In parallel to deficits found in spatial learning experiments, there is pharmacological evidence that a blockade of NMDA receptors interferes with place cell function. Place field stability is disrupted after intraperitoneal injection of the NMDA receptor antagonist CPP (98), suggesting a link between the ability of the mice to maintain stable place fields and their ability to retain spatial information. Although Kentros et al. (98) found effects of NMDA receptor antagonists on place field stability, they also found that CPP did not block previously formed spatial maps, nor did it block remapping in a new environment, suggesting that NMDA-mediated LTP may not be involved in the recall of previously formed maps or in the initial establishment of new place fields. However, these newly established fields are not retained, suggesting that LTP could be involved in the retention of these fields, at least in adult animals given an acute blockade of NMDA receptor function.

Experiments on genetically modified mice lacking NMDA R1 receptors in hippocampal area CA1 also have found spatial learning and place field deficits. As discussed in previous sections, these mutant mice perform poorly in the Morris water maze (53) and have decreased LTD and LTP. They also have both uncorrelated place cell firing and larger place field sizes (99). These results differ somewhat from the pharmacological experiments studying CPP-treated mice. Kentros et al. (98) found no effect of CPP on field size, whereas McHugh et al. (99) found enlarged field sizes in the NMDA R1 knockout mice, suggesting that the place cells in the knockout mice were less spatially selective than were those in wild-type mice. One possible explanation for this difference is that the deficits in spatial selectivity in NMDA R1 mice occurred because place cells become spatially selective during development. The NMDA R1 deletion occurred by approx 19 d after birth, which is when mice begin to explore their environments and thus when place fields might begin to form. That they failed to form spatially selective place fields may reflect the idea that spatial selectivity forms during development. The acute CPP treatment given by Kentros et al. (98) to adult mice would not have affected such development.

The parallels between NMDA receptor function and place cell function are striking and strengthen the argument that LTP is involved at some level in place cell mechanisms. A similar connection has been made between synaptic plasticity and place cell function in mice overexpressing a calcium-independent form of CaMKII [CaMKII Asp 286 (90)]. These mice have deficits in the Barnes circular platform maze. They show normal LTP when stimulation occurs at 100 Hz, but they show deficits in LTP when stimulation occurs at 5–10 Hz (62,100). This 5- to 10-Hz range of stimulation is similar to naturally occurring oscillations caused by theta cells, which are active in response to locomotion and also may encode some spatial information (92). Rotenberg et al. (90) found that CaMKII Asp 286 transgenic mice had fewer place cells, and those place cells that did develop had larger fields, lower firing rates, and less stability than wild-type place fields. They hypothesized that the inability to strengthen synaptic connections at low frequencies resulted in the observed place cell deficits. These experiments establish a strong correlation between LTP deficits and place cell deficits, although they do not establish LTP as a causal mechanism for any particular place cell function.

There is additional evidence that place cell deficits are correlated with long-term memory deficits. R(AB) mice, which show a selective impairment in L-LTP, also have impairments in place cell function (101). Rotenberg et al. (101) found that R(AB) mice had normal place cell firing rates and well-formed place fields, but these place fields were unstable over long

periods of time. However, with repeated exposure to the same environment, the place fields became more stable, suggesting that other signal transduction pathways may be recruited to counter the place field deficit.

In addition to maintaining place fields, LTP may contribute to spatial learning and the formation of cognitive maps by strengthening synaptic connections between those neurons that fire at the same time and therefore have overlapping place fields (44). Place fields that do not overlap will not fire contiguously and the strength of the synapse between them therefore will not change. Thus, the spatial distance between two fields may be represented by the strength of the synaptic connection between the neurons that give rise to those fields. What remains to be seen is what happens to synaptic strength between the synapses of two place cells that fire contiguously in one environment when the organism is placed in an environment where only one of those cells fires. The number of potential synapses in areas CA1 and CA3 alone is large enough to have unique connections in multiple environments, but any overlap of synapses in more than one environment may lead to degradation of the synaptic strength as a result of that synapse being differentially activated in different environments. Although some ideas have been put forward about mechanisms to deal with this issue (44), it is not clear how the problem will be solved.

More is being learned about the properties of place cells and their importance for certain forms of synaptic plasticity and learning, but several issues remain unresolved. One concerns whether place cells are involved simply in forming a map of space or whether they play a more active role in navigational processes. The hippocampus has been shown to be important not only for solving explicit spatial problems but also for learning about simple associations between a given context and a shock (57,58,63). It may be that place cells are important only for learning about the features of an environment, which would be necessary to solve context-based problems. Once a contextual representation is formed, place cells may work in conjunction with other systems to produce spatial navigation through an environment. One other type of cell that may contribute to a navigational system is the head-direction (HD) cell. Whereas the firing of place cells depends only on the position of the organism in space, HD cells respond only to head direction, independent of the organism's position in space. Unlike place cells, which may go silent in different environments, HD cells fire consistently in different environments, but their preferred directions may change (102). Although the preferred directions of a given set of HD cells may change in different environments, the angle between the preferred direction of any given two cells has been observed to be constant across environments (102). One result of such coordinated activation may be that the strength



between two synapses reflects orientation angles in the same manner as synaptic strength could reflect distances between place cell fields. This orientation, in conjunction with specific location coded by place cells, may form the basis of a system necessary to orient and navigate in an environment.

The recent work on genetically modified mice has allowed a connection to be made among place fields, LTP, and behavior. The recent advances in the study of place cells opens the floodgates to a host of important questions that, prior to the development of genetic techniques, may have been unanswerable. For example, assuming that LTP contributes to the formation and maintenance of cognitive maps, one would like to know how this map is read. Is it read in the hippocampus, where it is produced, or is the map read in other brain regions important to instigating behavior, but that do not have place cells, such as the prefrontal cortex (103,104)? Place fields do not seem to be modulated by the location of food reward in the environment (96), suggesting that place cells themselves do not code any information about the location of biologically significant stimuli. How, then, does an animal learn the location of food rewards in mazes? Where is this aspect of a cognitive map stored, and how might synaptic plasticity contribute to the linking of the location of reward stimuli to the brain areas responsible for processing rewards? These are important questions, and as more is learned about the respective contributions of synaptic plasticity and place cells to cognitive mapping, the answers to these questions will become clearer. The development of transgenic techniques to drive gene expression in restricted brain regions in an inducible fashion may be especially helpful for solving these unresolved issues.

## FUTURE DIRECTIONS

We have reviewed some electrophysiological, biochemical, and genetic studies linking changes in synaptic strength to learning and memory. These studies have provided strong evidence that synaptic plasticity plays an important role in learning and memory. Nevertheless, it remains to be demonstrated conclusively that synaptic plasticity is the cellular mechanism underlying learning and memory. With this in mind, we now turn to a discussion of some unanswered questions in synaptic plasticity research. The continued integration of more sophisticated behavioral studies with more molecular and genetic approaches will be needed to resolve many of these questions.

### *Aplysia*

In *Aplysia*, the argument in favor of a role for both postsynaptic LTP-like changes and presynaptic changes in neuronal excitability appears strong, especially for the cellular model of Pavlovian conditioning. Recent evidence

gathered in the reduced preparation makes a case for the involvement of postsynaptic mechanisms in learning (17,22), but the generality of these findings to the intact animal remains unclear. Indeed, the focus on the pre-synaptic and postsynaptic cellular mechanisms underlying learning has brought us back to where we started—the analysis of the behavior of the organism.

### *Contiguity, Synaptic Plasticity, and Learning*

Behavioral demonstrations of such important phenomena as blocking (3), conditional discrimination (28), and second-order conditioning (5) in *Aplysia* suggest that the repertoire of behavioral learning processes in *Aplysia* may be similar to those in vertebrates. There are, however, many other avenues to explore to further examine the extent of this similarity. One critical area of research that needs to be explored more thoroughly in *Aplysia* is the role that CS-US contiguity plays in establishing and maintaining learning. The past 30 yr of research and theory in the behavioral analysis of animal learning has been guided by several demonstrations showing that simple contiguity is neither sufficient nor necessary for learning to occur (105–107). The implications of such results have not been explored thoroughly at the cellular and molecular levels. Indeed, models of synaptic plasticity such as long-term facilitation and long-term potentiation rely on contiguity as the determinant of changes in synaptic strength. However, given that contiguity often fails to engender learning at the behavioral level, do these cellular models have any relevance to behavior?

One way in which associative theories of learning have been able to save the notion of contiguity is to assume that contiguity between a CS and a US will in fact lead to learning, unless other cues present on a conditioning trial, such as the conditioning context, are already strongly associated with that US (108). This competition among cues for conditioning has generated important insights about the learning process and has allowed contiguity-based theories such as the Rescorla–Wagner model to explain the situations in which contiguity fails to produce learning. Exploring the assumptions of successful contiguity-based models like the Rescorla–Wagner model may prove fruitful when developing theories of synaptic plasticity and when designing experiments at the cellular level.

### *The Structure of Learning*

An exciting area of behavioral research that is now beginning to be investigated at the cellular level involves determining the associative structure of the learning that underlies conditioning (5,109). Second-order conditioning has been a powerful tool for revealing this associative structure (110). In a second-order conditioning paradigm, a CS (A) is paired with a US for several

trials and then a novel CS (X) is paired with the original CS (A) for several trials. A test of X alone often reveals a conditioned response to X, despite X's never having been paired with a US. Why does the animal respond to X? There are two possibilities. One is that X has entered into a direct association with the response (stimulus–response, S–R, learning); the other is that when X is presented, the animal recalls a representation of A (stimulus–stimulus, S–S, learning), which, in turn, recalls a representation of the US. If it can be shown in *Aplysia* that responding to X is mediated through A, cellular theories of learning would have to be modified because they have focused almost exclusively on the sensory–motor synapse. A finding of S–S learning in *Aplysia* would suggest that synapses between sensory interneurons may be of critical importance in mediating behavior (5). Determining what role, if any, LTP might play in determining whether S–S or S–R learning occurs may help shed light on an important behavioral phenomenon from a cellular perspective.

### ***Hippocampal LTP***

Its ability to integrate information at the cellular, molecular, and behavioral levels makes the mouse hippocampus an ideal system to study because all the tools are in place to define the cellular mechanisms that underlie memory storage. What future directions in that field will be most fruitful for these studies?

### ***Appropriate Protocols for Inducing LTP***

Many fundamental questions remain about the appropriate protocols for inducing LTP. LTP often is studied in the hippocampus, but there are several areas within the hippocampus where LTP may be important for memory storage, including the Schaffer collateral, mossy fiber, and perforant pathways. The use of genetically modified mice in which LTP is selectively impaired at a subset of synapses within the hippocampal circuitry have recently underscored the important role of synaptic plasticity in hippocampal area CA1 (53,111,112). Many of these studies, however, have examined LTP in hippocampal slices, and one might get different results based on whether LTP is recorded in slices or in vivo, as revealed by the recent studies of Thy-1 knockout mice (112,113). Many questions will be engendered by the study of LTP in vivo, and there clearly is a need for more in vivo studies of LTP in mice, recording from a variety of synapses within the hippocampus. Given the differences observed between LTP in brain slices and LTP in living organisms, how can we be certain that mechanisms inferred from brain slices are the endogenous mechanisms controlling synaptic plasticity and memory storage?

On a behavioral level, also, there is a critical need to standardize the behavioral assays performed in different labs. Further, it is critical to ensure that behavioral studies are carried out and analyzed properly. In some Morris water maze experiments, for example, only the latency to find the platform during training is presented. To fully evaluate spatial learning and memory, however, other variables such as swim speed and the extent of thigmotaxis need to be analyzed. Importantly, performance on a probe trial, in which the platform is removed from the pool and the animal's swim path is recorded, needs to be determined. Because genetic background can have a dramatic effect on behavioral performance, this, too, needs to be standardized (114). Unfortunately, even if behavioral tasks are standardized, different labs may generate different results based on subtle differences in equipment or training protocol (121). It therefore also is important to use a variety of behavioral tasks that depend on similar underlying brain systems.

#### *Effects of LTP Reversal*

If LTP is an important cellular mechanism of memory storage, then the erasure of LTP following acquisition should lead to impaired performance. If one could "erase" LTP after learning has occurred, would this cause the animal to forget? Although this is an intriguing question, one of the reasons that it has not been addressed is that it is difficult to reverse LTP once it has been established. Most pharmacological treatments are active only when applied at or around the time of tetanus and have no effect when applied after LTP is established. One potential way of erasing LTP would be to genetically induce "depotentialization," an electrophysiological treatment (typically 5- to 10-Hz stimuli) that reduces LTP (115). If the molecular reagents that would "erase" LTP could be identified from the study of depotentialization, then reversible gene expression systems, such as the tetracycline-regulated system (116), could be used to activate expression of this LTP-erasing molecule just after learning a hippocampus-dependent task. Further, with the regional- and cell-type-specificity possible with genetically modified mice, LTP could be erased only in a subset of neurons within the hippocampus. If the erasure of LTP in a certain subset of neurons within the hippocampus impaired memory, then the link between synaptic plasticity and memory storage would be greatly strengthened.

Of course, discussion of the potential effects of erasing LTP begs the question of the effects of enhancing LTP. Would the enhancement of LTP enhance memory as a result of increasing synaptic efficiency and gene transcription, or would it impair memory perhaps due to saturation effects? Results that speak to this issue give an uncertain picture. Two recent articles have described genetically modified mice with enhanced LTP. PSD-95

mutant mice have enhanced LTP, but they exhibit spatial learning impairments (117), whereas mice lacking nociceptin receptors exhibit enhanced LTP and improved spatial learning (118).

### *Other Cellular Models of Memory Storage*

One major criticism of the study of the cellular basis of memory storage is that the field has been dominated by the hypothesis that changes in synaptic strength mediate memory storage (120). One of the reasons that LTP has been the focal point for so much research on synaptic plasticity is that there are few alternative cellular or systems models that might account for memory storage.

We have reviewed one of the systems properties of the hippocampus, the place cells that fire when an animal is located in a specific portion of the environment, and we have explored the relationship between these place cells and LTP. Studies examining this relationship have found that modulating LTP—either pharmacologically or genetically—leads to altered place cell properties. To distinguish between the role of synaptic plasticity and place cells in mediating memory storage, we need to expand the study of place cells in genetically modified mice, looking, for example, to see if place cell properties are normal in mice that behave normally but have impairments in LTP at a subset of synapses in the hippocampus (111,112).

What other cellular mechanisms might be involved in memory storage? One possibility is that rather than altering the strength of existing synapses, learning may lead to the formation of new synapses or may activate previously “silent” synapses by the insertion or activation of AMPA-type glutamate receptors. Such mechanisms do not necessarily preclude the involvement of LTP—those synapses that are potentiated may act as a short-term marker for the formation of new synapses (119). If this were the case, then erasing LTP after a critical period of time, as proposed earlier, would not affect memory because LTP may play a role only for a short time period after learning. Thus, although LTP may not be the cellular representation of the long-term store, it may be a critical step in establishing the growth necessary for long-term storage.

Another way in which memories might be stored at a cellular level is through changes in neuronal excitability, measured in terms of fEPSP-spike potentiation. In this model, neurons would be more or less able to fire action potentials, and this would be altered with learning. In a sense, this is reflected in (or forms the basis of) place cells because they are recordings of firing rates. The problem with this mechanism is that it would be hard to use in developing models for how neuronal firing could be regulated in an associative way.

Electrotonic changes in the way synaptic signals are integrated over distance in the dendrite may serve as another mechanism mediating long-term memory storage. In this way, the activity of individual synapses *per se* would not change, but the effective electrotonic distance of the synapses from the cell body and axon hillock would be altered. This would be accomplished by changing the active properties of dendrites, thus dramatically altering the ability of the neuron to respond to the activation of specific synapses. If this were restricted to specific branches within the dendritic tree, then this mechanism would mediate associative learning.

There clearly are many alternative cellular mechanisms that may drive memory storage. The introduction of a viable alternative to synaptic plasticity will be important not only for exploring alternatives to synaptic plasticity, but for establishing a theoretical basis for testing the limits of synaptic plasticity as a workable model for learning and memory.

Although many questions about the molecular and cellular mechanisms of learning and memory remain unanswered, the coordination of a variety of approaches—biochemical, genetic, pharmacological, electrophysiological, and behavioral—has generated a much richer understanding of the learning process than has ever been possible before. However, with this broad perspective comes the daunting task of assimilating these perspectives into meaningful explanations of learning and memory processes. Each of these approaches points to synaptic plasticity as the potential neurobiological building block for learning, and our study of the cellular and molecular mechanisms underlying learning and memory brings us back to the core problems that inspired us to investigate these issues in the first place—identifying the signal transduction pathways that underlie learning, the mechanisms by which learning modifies these pathways, and the way in which these modified pathways, in turn, influence memory and behavior.

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